SOME ANATOMICAL ASPECTS OF FRUIT DROP IN CITRUS

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I. INTRODUCTION

Autumn leaf fall and the drop of flowers, fruits and other plant organs are familiar examples of abscission, which is an important recurring phenomenon in plants. Addicott (2) held that the process of abscission plays an important role in the survival of the individual plant and the perpetuation of the species. He further elaborated on the subject as follows:

"1. Abscission permits the dispersal of seeds, fruits, and other reproductive structures.

2. Abscission reduces the rate of water loss, as in shedding of leaves under conditions of drought.

3. Abscission of excess young fruits permits the remaining fruits to attain greater size."

Lloyd (55) emphasized the important role of abscission in the multiplication of simple plants, such as algae, by a separation between contiguous cells; and in the breaking away of pieces of stems and leaves, and the shedding of leaves, in plants like Bryophyllum and Begonia, where they serve as a means of propagation. He described all these as a phenomenon different from the mere sloughing off of dead parts.

The abscission of flowers and the excessive drop of young fruits under certain circumstances, however, may not be desirable from the growers' point of view because of the economic factors.

In northern states where apple and pear are grown, observations have shown that normally less than ten percent of the apple blossoms which open in spring produce fruit. Many of the flowers are lost a

few days after the petals fall, and a large number of the partially developed fruit are shed during the next few weeks. A rather conspicuous drop, commonly called "June drop", occurs in June and July. Ordinarily an apple tree produces an abundance of flowers, but sometimes little or no fruit is harvested, practically all the fruit being lost during the "June drop", or before, in certain seasons.

The magnitude of the problems is such that it has attracted the attention of many workers seeking to develop some means to control the excessive fruit drop in deciduous fruits such as apple and pear.

In citrus, although the problem is not so severe as in apples, nevertheless under certain conditions it becomes quite pronounced and of great concern to the grower.

Citrus trees, like many other fruit trees, have the tendency to bloom heavily. Not all blooms that are borne by a tree set fruit, most of them being shed. Among the many causes, one may be poor pollination and failure of fertilization to take place. Coit and Hodgson (16) found that abscission of flowers and small fruits takes place more readily in the Washington navel oranges as compared to other less sterile varieties. This they attributed to the lack of fertilization in navel oranges.

It is well known, however, that a larger crop of young fruits is usually set by a citrus tree than can be brought to maturity. Developing fruit and seeds make a great drain on the stored energy of the tree, and as a result the trees may shed a large proportion of their young fruits about the time the petals fall or soon thereafter.

Generally sufficient fruit remains after this fall to produce a satisfactory crop, but this is not always the case. The dropping of fruits continues to some extent throughout the growing season. A second heavy fruit drop occurs usually during the months of May and June, which as in apples is popularly called the "June drop". The number of developing fruits lost in this drop usually does not equal the number lost in the first one. However, the comparatively bigger size of the fruit lost during the "June drop" makes it quite conspicuous. Under certain conditions this drop becomes so pronounced that the remaining crop borne by the tree becomes altogether uneconomical. The excessive fall of young fruit is very exasperating to growers, who would undoubtedly make much greater profits if some way could be devised to prevent that part of the drop which is in excess of the normal and necessary amount.

A third period of increased abscission of fruits coincides with their losing green color and turning yellow, late in the growing season. This wave of fruit drop has been termed the "preharvest drop". This drop is also often of great concern to the grower, because a good proportion of fully developed and almost mature fruit may be lost shortly before harvest time in this way. The importance of the problem has been equally realized in Florida and California, and some important work has been done in the direction of controlling such drops through the use of growth- promoting substances (82, 87).

During the course of such studies Gardner et al. (25) brought out that the delay in fruit drop is due to delay in formation of

the abscission zone. They have emphasized the need of an anatomical study of this zone, in order to fully understand the mechanism of fruit fall so that the growth-promoting substances may be used more advantageously.

The present study, therefore, was planned to investigate some of the anatomical aspects of fruit-drop in citrus, with particular reference to the Hamlin orange which is one of the important, early-maturing varieties grown in Florida.

II. REVIEW OF LITERATURE

Review of the literature reveals that the importance of the premature fruit drop and leaf fall in several plant species was recognized more than one hundred years ago. However, the work reported by Parkin (73), Tison (89), and Lee (52) makes the basis for present knowledge on the subject. Earlier work was mainly confined to the better understanding of the anatomy of the plant and the abscission phenomenon, without any effort to use this knowledge about abscission to regulate this phenomenon.

The work of Coit and Hodgson (16) and Hodgson (40) on citrus, of Lloyd (55,56) on cotton, and of MacDaniels (60) and McCown (63, 64, 65) on apple, constitutes a great contribution towards a better understanding of abscission in the plants with which they worked.

A. Anatomy of Abscission

The study of the body of literature on the subject reveals that the fall of fruit, leaves, and other plant organs is brought about by the separation of a special group of cells known as the abscission layer. The work of Coit and Hodgson (16), Gawadi (29), Hodgson (40), Laurie and Duffey (51), Lee (52), Lee and Carolus (53), Parkin (73), Sampson (77), and Scott et al. (70) is in agreement on this point. Working with different plants, they all reported that with some exceptions, abscission is restricted to well defined regions, usually found at the base of organs as, for example, the base of the petiole , the base of the fruit, and the base of the

internode. Abscission zones usually have a diameter smaller than that of adjacent portions and so are often marked externally by a constriction. The cells in the abscission zone are generally smaller than those of adjacent regions, intercellar spaces are lacking, and vascular elements are reduced in amount and shorter. The fibers associated with vascular tissues are usually absent. In the abscission zone proper, the parenchyma shows no signs of lignification and only traces of suberization. Adjacent parenchyma often shows a relatively high degree of lignification and suberization. The above workers conclude that considering everything, these characteristics suggest that the abscission zone is a region where development toward maturity has been arrested.

Lee (52) studied the abscission phenomenon in a number of dicotyledons and reported that the chief modification at the leaf base in connection with leaf fall is the formation of a separation layer which is produced from existing cells, with or without division. The leaf separates from the stem by the disappearance of the middle lamellae of the cells of the separation layer and the subsequent rupture of the sieve tubes and vessels of the leaf-trace at that level. A lignified layer may or may not be present, but a protective layer is invariably produced either before or after leaf fall. The mode of formation of the protective layer is (1) by ligno-suberization of the cells of the leaf base with or without irregular division, and (2) by ligno-suberization of cells produced by the continued division of a regular cambium.

The investigation into foliar abscission in Washington navel oranges under California conditions, conducted by Hodgson (40), showed that in young material there is no histological distinction in the cells which would enable one to fix upon the ten to eighteen layers destined to function later as the abscission zone. In older material, both at the base of the terminal leaflet and at the base of the petiole, the cells in the abscission zone are smaller and differ in general appearance from those on either side. Actual separation occurs one or two tiers of cells proximal to the distal end of the abscission zone, that is, one or two layers below the upper end of this zone. Ordinarily only one or two layers of cells are involved in the separation process, but occasionally as many as four or five may take part. However, it is seldom that the cells of more than one tier actually complete the process.

According to Laurie and Duffey (51), working with gardenia, the flower bud may abscise at the junction of the pedicel and stem or it may abscise at the junction of the corolla tube and pedicel.

The abscission zone in gardenia appeared as a zone of cells four to six tiers thick in the cortex and six to eight tiers thick in the pith. This zone began in the epidermis and extended across the cortex to the vascular tissues. It did not extend across the vascular tissues, but it did extend across the pith. The zone apparently originated independently in the epidermis and cortex and simultaneously in the pith. The cells in the abscission zone had thicker cell walls and were slightly more rounded than the cells outside of this zone. The cells in the tissue of the ab-

scission zone were parenchymatous, as were the cells of the surrounding tissue. The lamellae of these cells were prominent due to the change of pectic compounds in the middle lamella of the cell walls. The separation of cells started in the epidermis and extended into the cortex for a short distance. This separation involved one to two rows of cells and appeared to be due to the dissolution of the primary cell wall and the middle lamella. The secondary cell wall did not dissolve and was seen as a distorted piece of tissue clinging to the tissue yet intact.

Gawadi (29) concluded that shedding of leaves and other structures frequently was preceded by localized cell division at the base of the leaf or other organ being shed. This cell division results in a thin transverse layer, from a few to several cells thick. He added that the abscission layer is not causally related to these organs, because abscission not only occurs in species which do not have an abscission layer but can be experimentally induced before the abscission layer develops in those species which ordinarily possess it. Unlike many of the other workers, Kendall (47) did not observe any cell division or cell elongation accompanying abscission in many species of Solanaceae. Similarly Dutt (19) reported no evidence of cell division prior to abscission of bolls in cotton. McCown (64, 65), working with apple, found that the abscission of the flowers and of immature fruits was preceded by differentiation of an abscission layer, whereas the abscission of mature pedicels was initiated independently in the pith and cortex

and not preceded by cell division.

In certain species a modified type of abscission of different plant organs has been reported. Addicott (1), working with guayule, and Berkeley (8), working with several species of oaks, reported such a modified type of abscission.

In guayule (1) the abscission layer is not directly involved in the separation of the leaf from the stem. Separation is mechanical and occurs after the leaf dies by a break which passes through the weak abscission zone at the base of the leaf. There is no indication that physiological action such as the digestion of the middle lamella or of whole cells is involved in the freeing of the leaf. Protection for the stem is provided by the abscission layer and the periderm which develops beneath it. The abscission layer forms at the leaf base in cortical parenchyma, resin canal plugs and within the leaf trace. It is continuous with the periderm of adjacent regions of the stem and is well suberized before the leaf falls.

A similar type of modified abscission has been reported in several oak species (8) where the leaves die at the end of the growing season but remain attached to the twigs until the beginning of growth the following spring. It has been suggested that the fall of the dead leaves on resumption of growth is due to the development of a suberized protective layer.

B. Physiology of Abscission

In recent years the physiological aspect of the abscission of different plant organs has also received much consideration. It has been shown that the formation of abscission layers in leaves as well as in flowers and fruits is not merely a mechanical rupture, but results in chemical change of the cell walls in the abscission zone. Consequently any condition, internal or external, which retards or accelerates the rate of physiological activity would naturally affect the rate of abscission.

Brown and Addicott (10), Dutt, (19), Facey (22), Hodgson (40), Kendall (47), Lloyd (57), McCown (65), Ratsek (75), and Sampson (77) showed that walls of all the cells of the abscission zone are altered chemically during abscission. The greatest degree of alteration takes place in the cells which are actually involved in separation, in which alteration by hydrolysis proceeds so far as to procure the complete digestion of a part of the primary and secondary walls. This allows a great extension of the tertiary walls and their separation from each other and from the only partially altered primary and secondary walls of the next distal tier of cells. The separation layer is composed of one to several tiers of cells depending upon the plant studied.

These workers further added that the abscission of different plant organs is associated with the conversion of cellulose into pectose, which is further transformed to pectin and pectic acid, leading to the formation of an excess amount of pectic acid over

that of the available calcium sufficient to maintain the solidity of the middle lamella of the cell walls of the abscission layer. These pectic compounds absorb excessive amount of water and become gelatinized. Abscission then taken place easily.

It was also noted by most of the above writers that prior to the initiation of abscission, the tissues on both sides of the abscission zone are plentifully supplied with starch, which is either withdrawn or used before the process begins. At this time the cells of the abscission zone contain considerable starch while the tissues on both sides contain very little. The starch content decreases as the process goes on, but even at complete separation the abscission cells contain notable quantities of it.

Kendall (47) states that the dissolution of the middle lamella, which ultimately results in the abscission of the tissue attacked, is brought about by enzymes.

C. Factors Affecting the Abscission Phenomenon.

It has been reported by several investigators that there are numerous climatic and environmental factors having strong influence on the abscission phenomenon in plants.

The work of Baird and Laurie (5), Bradbury (9), Carns and Addicott (12), Coit and Hodgson (15), Furr et al. (23), Garner and Allard (28), Goodspeed et al. (30), Gustafson (31), Hasek (36), Heinicke (37, 38), Hodgson (41), Jester and Kramer (43), Keyes (46), Kraus and Kraybill (48), Luckwell (59), Matzke (61), Murneek (67),

Olmsted (72), Radspinner (74), Smith (89), and Srivastaya (86), indicates that mechanical shock, lack of fertilization, excessive amount of certain gases, attack by insects or diseases, abnormal temperatures, desiccation, genetical constitution of the plant, length of photoperiod, internal physiology of the plant, and nutrient supply are some of the many factors which may have a strong bearing on the rate of abscission of plant organs.

1. Water Relations

The work done by Haas (34) and Hodgson (41) on water relations and abscission in citrus, revealed that an abnormal water relation develops periodically in citrus foliage and in the young fruits during the hot growing season in drier regions of California. It has been shown that leaves are able to withdraw water from fruits, during period of high temperature and hot, dry winds. The water deficit thus brought about serves as a stimulus and causes abscission of young developing fruits, or may interfere greatly with the development of the fruit.

2. Temperature Relations

Some investigators have reported that abnormally high air temperature or sudden changes in temperature act as an important factor in causing abnormally heavy drop of fruits.

According to Baird and Laurie (5), Hasek (36), Keyes (46), and Radspinner (74), the rate of abscission of different plant organs is enhanced when the air temperature is extremely high and the relative humidity low. Such conditions are more favorable for

excessive transpiration and result in a water deficit in leaves and fruits.

3. Oxygen Supply

Laboratory experiments conducted by Carns and Addicott (12) showed a positive correlation between rate of abscission and oxygen level. However, rate of abscission did not show any increase above an oxygen level of 40 percent. They observed the retardation of abscission in excised abscission zone kept under water. However, normal rate of abscission was obtained if oxygen was continuously passed through the water, demonstrating that the water effect was due to its interference with the availability of oxygen. It was concluded therefore that the cells of the abscission zone must be able to carry on respiration in order to function in abscission.

4. Photoperiod

Among various other environmental factors, the influence of the photoperiod on the rate of abscission has been considered important. Garner and Allard (28) suggest that a gradual transition from the long days of summer to the short days of early autumn is required to induce abscission in leaves of Soybean plants.

Jester and Kramer (43) reported that beech, red maple, Southern red oak, black locust and chestnut oak, both inside and outside the greenhouse, retained their leaves longer with continuous illumination and long days than with normal length of day.

Matzke (61) found eight street trees of Acer platanoides showing prolonged autumnal retention of leaves near electric lights.

However, he did not find any convincing effect of street lights on leaf retention in *Acer saccharinum*.

Olmsted (72), working with sugar maple, concluded that loss of green color and leaf abscission under natural conditions are conditioned by the decreasing autumnal photoperiod. He found that in general the amount of delay was positively related to length of constant photoperiod. A change from 16 to 8 hours of photoperiod in December accelerated senescence and abscission as compared with plants remaining on a 16-hour photoperiod.

5. Internal Physiological Condition of the Plant

The physiological condition of the plant has an important controlling effect on the various metabolic processes going on within the plant tissue.

The work of Cameron (11), Kraus and Kraybill (48), and Smith (84) suggests that quite likely a high level of nitrates in plant tissues favors the development of the abscission layer, whereas a relatively higher carbohydrate content makes for continued development of the vascular strands of the pedicels and the strengthening of their connection with the fruit spurs. However, a very marked abscission of tomato fruits and blossoms occurred when the carbohydrate content was very high under conditions of very low nitrate levels.

6. Nutrient Supply

Many workers consider that nutrient deficiencies may cause abscission. A nitrogen deficiency at blossoming time is one of the

most common causes of excessive abscission. Phosphorus deficiency may also cause abscission of flowers or young fruits. Zinc deficiency is apt to cause many flowers to fall before they fully open. However, results obtained by Dickson (17) showed that a low amount of nitrate in the soil resulted in a lowered percentage of fruit drop in McIntosh variety of apple. Srivastava (86), working with pears, reported that the main cause of "June drop" did not appear to be lack of fertilization, since plenty of apparently healthy seeds were found in most of the fruits dropped. The size of the individual fruit was found to be correlated with its length of life on the tree--the smaller the fruits, the shorter their life, or the larger the fruits, the longer they remained attached--thus suggesting that the dropping of fruits was due to competition for nutrients.

7. Fertilization

Proper fertilization is considered in most fruits as essential for set and development. Large numbers of pomaceous fruits fall from the trees before reaching maturity, due to the abortion of the embryo. Abortion of the embryo has been attributed most frequently to one or more of such conditions as self- or cross-sterility, lack of pollination due to unfavorable weather conditions during the blossoming period, lack of fertilization due to incompatability or to unfavorable temperatures for pollen-tube growth, improper nutrition of the tree, and competition of individual blossoms for nutrient materials.

Heinicke (37, 38) and Bradbury (9), working with apple and sour cherry respectively, laid emphasis on proper pollination and fertilization as conditions for the development of fruit.

In sour cherry, three abscission waves were recognized. The first one affected fruits with shriveled ovules, indicating either that no fertilization had occurred or that the zygote had aborted immediately. The second and third drops consisted of partially developed fruits in which the embryo and endosperm had degenerated following fertilization and initial development.

8. Pests and Diseases

Mechanical or pathogenic injuries are also reported to have an important influence on the abscission phenomenon.

Coit and Hodgson (15) observed that the "June drop" of navel oranges under California conditions is often brought about by a fungus of the genus *Alternaria*. Infection occurs in the blossom end and, on account of the peculiar structure of the navel orange, is able to persist. The extremely high transpiration rate in dry regions brings about a daily reversal of the sap current which transfers the poison produced by the fungus back through the joint in the pedicel, causing it to weaken and abscise.

D. Hormones and the Abscission Phenomenon

It has been established by many workers that the premature drop of fruit may be due to a lack of certain growth hormones which are produced by the fertilized ovule and translocated downward

to the abscission zone of the pedicel. Similarly the fall of the bladeless petiole is considered to be due to the absence of certain growth hormones which are synthesized in the leaf blade and transported downward to the abscission zone of the petiole.

As found by LaRue (50), abscission always resulted when the development of the abscission layer was not inhibited by hormones produced in the leaf blades. He found that if the blade of a leaf is cut off, leaving the petiole of the leaf attached to the stem, the petiole will soon abscise; but if indole-acetic acid is applied to the cut end of the debladed petiole, abscission is delayed. This suggest that auxin from the blade is the effective agent inhibiting abscission. The evidence suggests that abscission of an organ is inhibited as long as its auxin supply is high, and that abscission is initiated when its auxin supply becomes low, relative to the previous level in the organ.

The work of Luckwell (59) suggests that fruit drop in the apple is under the control of a hormone produced in the endosperm of the seed, and that the successive waves of fruit abscission are correlated with temporary deficiencies in hormone production, resulting from developmental changes in the endosperm.

Loomis (58) stated that in case of failure of pollination after anthesis little or no auxin is produced in the ovary. Then growth ceases and the ovary will generally abscise. One manifestation of this auxin response after fertilization is the inhibition of abscission layer formation, hence the organ is retained on the stalk.

Hall (33) concluded that the natural cause of abscission is

found to be the balance between auxin and ethylene in petiole or pedicel. His results suggest that the relative balance of ethylene to auxin determines the amount and rate of abscission effected.

Based on the foregoing consideration synthetic growth-regulating substances have been used to replace the function of a naturally produced hormone. Some have been commercially used to prevent the pre-harvest drop of fruit.

Addicott (3), Batjer and Marth (6), Edgerton (20), Erickson (21), Gardner _et al._ (25), Gardner and Cooper (27), LaRue (49, 50), Livingston (54), Marth _et al._ (62), Murneek (68), Murphy (60), Myers (70), Shoji (81), Weintraube (92), Whiting and Murray (94), Zielinski _et al._ (98), and many other workers have used various synthetic growth-regulating substances for control of flower or fruit abscission with varying degrees of success.

Beal and Whiting (7) reported that plants of _Mirabilis jalapa_, when decapitated and treated with 2% indoleacetic acid in lanolin on the cut surface of the stem, showed continued growth of the internodes and the entire absence of abscission or of an abscission zone at the bases of the internodes.

Gardner (26) reported that naphthaleneacetic acid and naphthalene acetamide could be used to lessen materially the drop of Pineapple oranges in Florida, but the high concentration and the necessity of early application discouraged the use of the method commercially.

Reece and Horanic (76) similarly showed that sodium salts of 2,4-D, and the triethanolamine salt of 2,4,5-T, at a concentration of 25 ppm. significantly reduced pre-harvest drop of Pineapple oranges. However, these compounds were not found effective in reducing fruit drop of Valencia oranges under Florida conditions. This is not in line with the results reported from California by Stewart et al. (87, 88), who found that by spraying Valencia orange trees bearing mature fruit in May with a 2,4-D derivative (the diethanolammonium salt), at a concentration of 24 ppm., as much as 78% of the normal pre-harvest drop could be prevented.

Sites (82), working with Pineapple oranges in Florida, summarized his work as follows:

"The triethanolamine salt of 2,4,5-trichlorophenoxypropionic acid applied as a spray to Pineapple orange trees resulted in a marked reduction in numbers of fruit lost due to pre-harvest drop.

"A concentration of 20 ppm. of the acid appeared to be adequate to give good drop control. Ten ppm. when used in combination with 1-5 pounds of urea per 100 gallons also resulted in satisfactory drop control.

"Time of application within a reasonable period does not appear to be critical. Significant reduction in percentage dropped fruit was obtained from sprays applied as late as February".

It is clear from the foregoing statements that the effectiveness of growth-regulating substances on the retardation of abscission varies with the substances used and the organ treated. Hitchcock and Zimmerman (39) stated that the results obtained by using

only one substance may vary with different trees of the same variety and even with the different branches of the same tree.

E. Summary

The study of the literature cited here clearly reveals that in most plants, the abscission of different plant organs is closely associated with the formation of a special layer of cells, popularly known as the abscission zone. Actual separation occurs by the dissolution of middle lamellae in one or two tiers of cells in this zone.

Various climatic and environmental factors have been reported to influence abscission in plants. Under certain conditions abscission of leaves, flowers, or fruits becomes so acute that growers are deprived of any harvest. Recent results obtained by the use of growth-regulating substances warrant hope that through careful study of the abscission phenomenon in economically important plants, field control of abscission will be made possible.

III. METHODS AND MATERIALS

A. Materials

1. Material for Anatomical Studies

Three apparently uniform trees of the same age of Hamlin variety of sweet orange, budded on sour orange stock, were chosen in the young citrus grove at the University of Florida, Gainesville, Florida. These trees have been growing under uniform conditions of orchard management since their planting in 1949.

The collection of the material for anatomical studies was started with the emergence of the bloom in 1956, while in the 1955 season it was started with petal fall. The samples were collected at weekly intervals until the end of September in both seasons.

Five pedicels along with some stem portions were taken from each experimental tree at each sampling in the 1955 season. Fifteen pedicels each time thus made a sample. However, in the 1956 season, five terminal and five subterminal lateral pedicels were collected from each experimental tree. Thus each time fifteen terminal and fifteen lateral pedicels made a sample. Collection of terminal and lateral pedicels separately was started to find out if there is any difference between the rate of abscission in terminally and laterally located fruits.

In all, 24 samples were collected and preserved for histological studies in the 1955 season, making a total number of 360 pedicels for examination.

A total of 30 samples were collected in the 1956 crop season. During this year, the collection of material was started at a much earlier floral stage and the first sample was gathered on February 15, 1956. Thus a total of 450 terminal and 450 lateral pedicels were collected during this season.

2. Material for the Study of Hormone Effect on Abscission

The same precautions were taken in the selection of pedicels as were exercised in the selection of the material for the anatomical studies. The samples were collected on September 15, 1956. Ten pedicels or explants per treatment made a sample. Sucrose, 2,4-D, indoleacetic acid, and leaf and seed extracts of Hamlin oranges were used for treating these explants.

B. Methods

1. Anatomical Studies

The collection of samples for histological studies was at random. Samples were collected according to the procedure already mentioned. However, care was taken that the pedicels were collected of fruits of almost the same size located at the same height on the tree, equally exposed to light and well distributed around the tree.

The standard techniques recommended by Chamberlain (14), Johanson (44), and Sass (78) were adopted for histological studies, with slight modifications wherever found necessary.

The most common fixitive fluid, formalin-acetic-alcohol, was employed for killing, fixing, and preserving the material.

Since the material was killed and fixed in formalin-acetic 50% alcohol solution, the material was rinsed in several changes of 50% alcohol to remove all the traces of fixitive fluid in order to avoid inconvenience at the time of staining. Normal butyl alcohol was used as dehydrant. By substituting butyl alcohol for ethyl alcohol and xylene, the process of dehydration and paraffin infiltration was much simplified. Xylene was found to harden the tissues excessively, while this defect was overcome by use of N-butyl alcohol for dehydration. Sections from 10 to 15 microns in thickness were cut, after the material had passed all the stages of the standard paraffin imbedding method.

However, this method could be used only for tender material collected from February through early May. After that, tissues became so hard that the paraffin method was either abandoned in favor of the celloidin method, or the material was softened in hydrofluoric acid before embedding it in paraffin, as recommended by Jeffrey (42). When the celloidin method was used, the technique as outlined by Wetmore (96) was followed.

It was found much easier and more convenient to cut hard pedicels late in the season fresh, with the help of a sliding or table microtome. For the most part, therefore, hard material was cut fresh by using a sliding microtome. The toughness and size of the pedicels made it possible to clamp such material directly in the sliding microtome holder, even without the use of any sort of pith.

Safranin-fast green combination of stains was found to be most

satisfactory, since good differential staining resulted. However, Delafield's hematoxylin was used for slides selected for microphotographs.

2. Methods of Treating Explants with Different Materials

It was not found possible to spray the experimental trees with different materials to see their effect on fruit abscission, consequently the method as recommended by Addicott et al. (3) was adopted for such studies in the laboratory. This was the use of explants which were essentially pieces of pedicels one centimeter long, including part of tissue where abscission usually takes place.

The explants were treated by immersing them for five minutes in different concentrations of aqueous solutions of sucrose, 2,4-D, indoleacetic acid, and in various strengths of leaf and seed sap extracted from the leaves and seeds of Hamlin oranges. The sap was extracted from leaves and seeds by use of a hydraulic press.

The treated explants were then mounted on 4 percent agar in petri dishes. After the agar had set, an inch-wide strip of it was removed along a diameter of the plate, leaving a narrow bridge of agar across one end to hold the agar in place. Ten explants per treatment were mounted on the agar of each plate, a part of each explant overhanging the central channel (Figure 1).

Ten explants in each experiment were mounted without any sort of treatment or water immersion and served as control treatment.

The plates were kept under daily observation for 14 days to note the evidence and progress of the abscission.

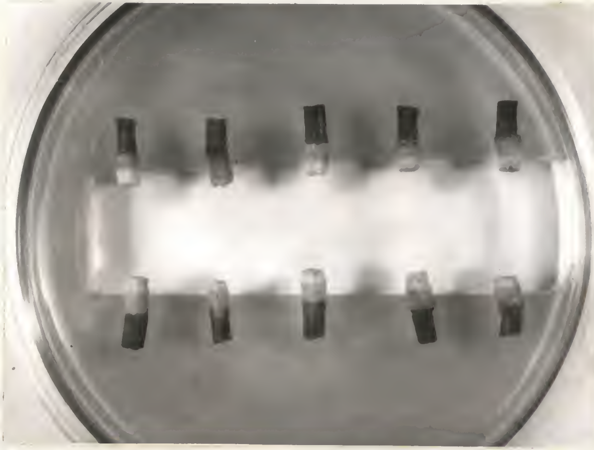

Fig. 1. Petri dish showing the explants mounted on agar.

3. Objectives of Investigation

It was proposed to study the following anatomical and physiological aspects of abscission of fruits in Hamlin variety of orange from the material thus collected.

a. Study of the abscission zone structure.

b. Study of the separation process.

c. Study of the periodicity of the abscission phenonmenon.

d. Study of abscission of fruits, as influenced by relative position on the stem.

e. Study of the effects of different hormone treatments of explants on abscission.

IV. RESULTS

A. Results of Field Observation

Through the flowering, fruit development, and fruit ripening season of 1956, the experimental trees were kept under visual observation. The flower buds started swelling in the first week of February 1956. During the second week flowers started opening and bees were active. Three waves of fruit drop were quite noticeable during the season. The first coincided with petal fall in early March, the second with the onset of hot dry weather, and the third synchronized with color-break in the fruit.

In the early stages of fruit development the abscission of minute fruit was mostly found to be at the base of the pedicel. By the first week of May, some fruit also abscissed at the base of the ovary.

After the second week of May the abscission was mostly at the base of the developing ovary (Figures 2 and 3). The fruits lost during these drops were found somewhat undersized and showing yellowing near the base of ovary (Figures 4, 5 and 6).

Sometimes it was also noticed that pedicels remained attached to the main branch after the abscission of young fruit (Figure 7).

The drop which occurred with petal fall generally consisted of pistils with distorted styles. The fruit lost in the last drop for the most part consisted of fruits showing splitting and fungal attack. The spores of Alternaria citri isolated from such fruits are shown in Figure 8.

Fig. 2. The early abscission, coinciding with petal fall, was mostly near the base of the pedicel. (March 15, 1956)

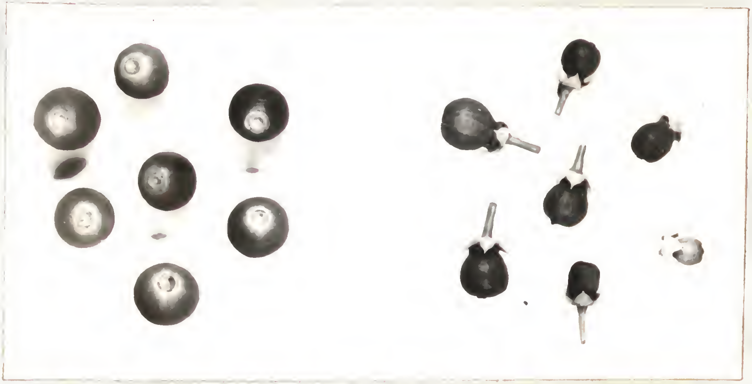

Fig. 3. On the right, small fruits abscised near the base of the pedicel (April 2, 1956). On the left, fruits abscised at the base of the developing ovary (April 28, 1956). Actual size of fruits is shown in this figure.

Fig. 4. Normally developing fruit. The undersized one is more prone to abscise.

Fig. 5. The smaller fruit showing partial separation, pointed out by an arrow, was light yellow in color as compared to other dark green, normally developing fruit. (April 28, 1956)

Fig. 6. Fruit showing smooth break at the base of the ovary with yellowing.

Fig. 7. On the right, pedicels which remained attached to the branches after the fruit fall. On the left, pedicels which also fell a few days after fruit fall.

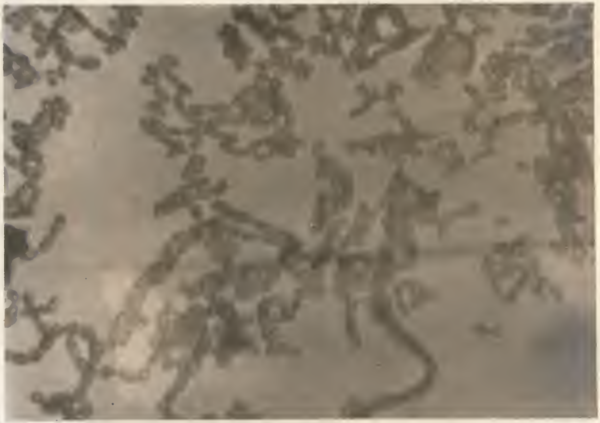

Fig. 8. <u>Alternaria citri</u>, showing spores borne in chains.

B. Results of Histological Studies

The permanent slides prepared according to the techniques previously described were examined to ascertain the abscission zone and to study its structure.

In order to understand the modifications found in the abscission zone, an understanding of the pedicel structure seems necessary (Figures 9 and 10). A normal pedicel consists of the following tissues.

Pith - centrally located pith is largely composed of large, thin-walled parenchymatous cells.

Xylem - surrounding the pith is the xylem tissue, composed largely of trachae or conducting vessels. In the primary xylem, adjoining the pith, there are also scalariform, ringed and spiral vessels. The wood fibers taper gradually and usually have a wedge-shaped end. Scattered more or less singly or in rows among the fibers and the vessels are the wood parenchyma cells. Xylem tissues are intercepted at intervals by radial rows of parenchyma, which are one to three cells broad tangentially.

Cambium - A thin walled layer of meristematic cells, which separate xylem and phloem.

Phloem - as described by Webber and Fawcett (91), in early stages of development phloem consists of parenchyma, sieve tubes and companion cells. Later phloem fibers are formed adjacent to the inner edge of the cortex. Phloem fibers are closely crowded, their lumina being nearly obliterated, while their walls are thick and lignified.

Phloem paranchyma is abundant, cells being nearly cubical with walls irregularly thickened. In the band nearest the cambium, sieve tube elements are easily distinguished from parenchyma cells by means of their large central vacuoles, sieve plates, and accompanying companion cells. The phloem elements are intercepted by phloem rays, which may be uniseriate or multiseriate.

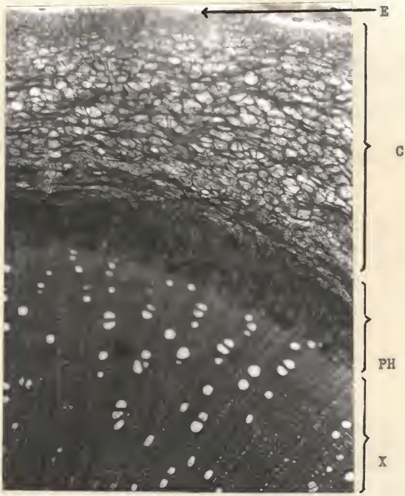

Fig. 9. Cross section of a mature Hamlin orange pedicel showing X (xylem and cambium), PH (phloem), C (cortex), E (epidermis).

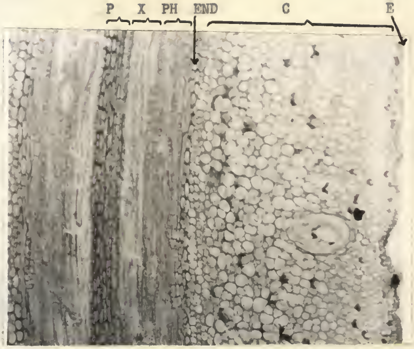

Fig. 10. Longitudinal section of orange pedicel, showing P (pith), X (xylem and cambium), PH (phloem), END (endodermis), C (cortex) and E (epidermis).

Fig. 11. Longitudinal section through basal end of a pedicel, showing reduction in the diameter, at level corresponding to abscission zone.

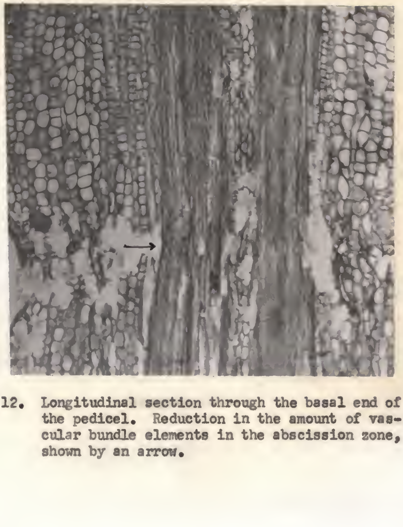

Fig. 12. Longitudinal section through the basal end of the pedicel. Reduction in the amount of vascular bundle elements in the abscission zone, shown by an arrow.

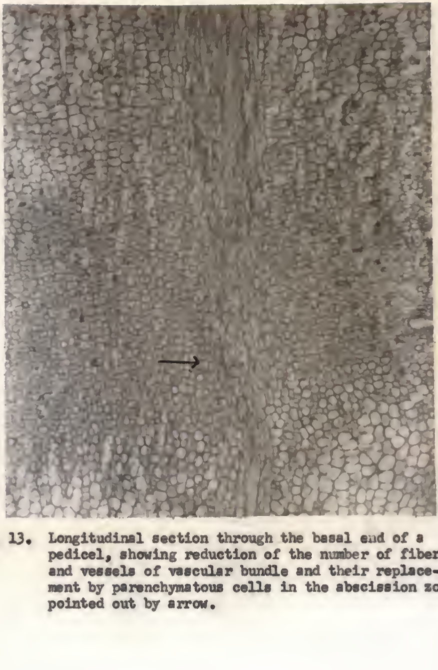

Fig. 13. Longitudinal section through the basal end of a pedicel, showing reduction of the number of fibers and vessels of vascular bundle and their replacement by parenchymatous cells in the abscission zone, pointed out by arrow.

Fig. 14. Longitudinal section through the basal end of a pedicel, showing at E, the modifications of epidermal cells in the abscission zone. VB (vascular bundle) elements are seen less affected by abscission process.

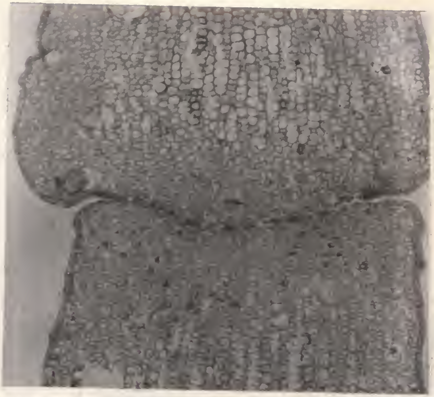

Fig. 15. Longitudinal section through the basal end of a pedicel. There is no hard tissue in the pedicel at this stage of development. The abscission layer cuts through ground tissue alone.

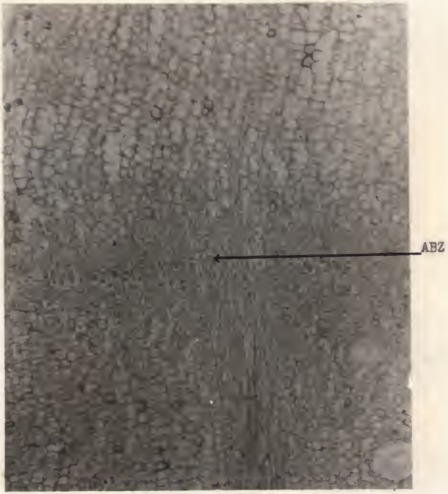

Fig. 16. Longitudinal section, showing abscission layer formation near the base of the pedicel in the early stage of fruit development (March 1, 1956). Abscission zone shown as ABZ.

Pericycle - As seen in cross section, a circle of cells is found between phloem and cortex. The cells in this ring are much elongated and have thick walls.

Cortex - cortex is chiefly composed of thin-walled parenchymatous cells. However, several layers of cells near the epidermis are of collenchyma type.

Epidermis - epidermis is composed of a single layer of almost isodiametric cells. The epidermal layer is intercepted by stomata. In mature peduncles, the outer walls of epidermal cells become cutinized.

The modification of the normal anatomy in the abscission zone, as observed, was as follows:

a. A reduction in the diameter of the pedicel corresponding to the abscission zone near the base of the pedicel (Figure 11), appearing even prior to anthesis.

b. A reduction in the number of fibers and vessels of the vascular bundles in the abscission zone and an increase of parenchymatous cells instead (Figures 12 and 13).

c. The modification of epidermal cells, corresponding to the abscission zone to form cushions of elongated cells which separate readily in the abscission process (Figure 14).

1. The Structure of the Abscission Zone

In citrus, early in the season, the abscission of flowers and of small fruits was ordinarily found to take place near the base of the pedicel. In immature pedicels, the abscission layer cuts through the parenchymatous tissues. The vascular tissue of the pedicel at this stage is undifferentiated procambium, with occasional protoxylem strands (Figures 15 and 16).

No cell division prior to abscission could be found. In young material it was difficult to distinguish the abscission zone from the adjoining tissues on both sides, because there was no histological distinction. However, cells in the abscission zone responded

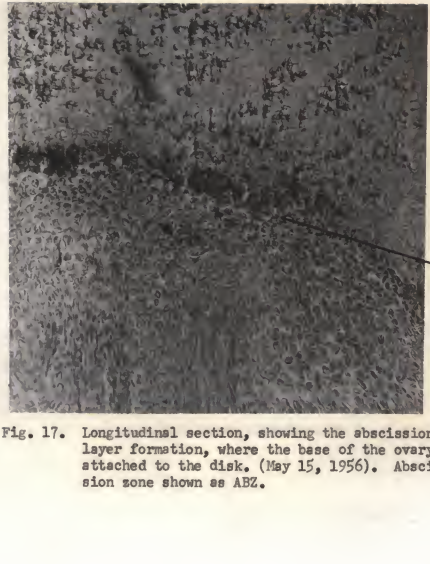

Fig. 17. Longitudinal section, showing the abscission layer formation, where the base of the ovary is attached to the disk. (May 15, 1956). Abscission zone shown as ABZ.

Fig. 18. Basal part of the same pedicel as shown in Fig. 17. Arrows indicate the development of the abscission layer at the basal end as well.

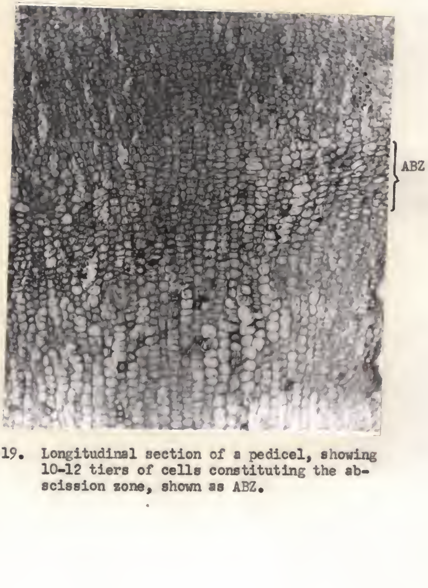

Fig. 19. Longitudinal section of a pedicel, showing 10-12 tiers of cells constituting the abscission zone, shown as ABZ.

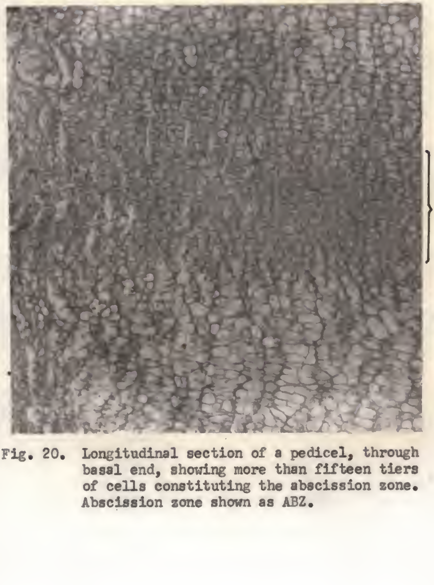

Fig. 20. Longitudinal section of a pedicel, through basal end, showing more than fifteen tiers of cells constituting the abscission zone. Abscission zone shown as ABZ.

ABZ

Fig. 21. Longitudinal section through the basal end of a pedicel, showing the small, brick-shaped, cells of the abscission zone. Abscission zone shown as ABZ.

differently to certain dyes used to locate this zone.

In accordance with the results reported by Lloyd (57), the cells in the abscission zone assumed a delicate greenish hue when stained with potassium iodide-iodine. On dilution with water this greenish hue was replaced by a light, pale blue tint.

As already stated, in immature material, collected at the beginning of the season, abscission always took place at the base of pedicel. However, from early May, the point of abscission was mostly found where the base of the ovary is attached to the disk (Figure 17).

In some cases, the abscission zone in mature pedicels was located both at the base of the ovary and at the base of the pedicel (Figures 17 and 18). However, the process of abscission probably is not completed at the base of the pedicels, due to formation of strengthening tissue. Field observation revealed that many pedicels remain attached to the main branch after fruit fall.

The abscission zone was found to be somewhat indefinite in its limits, usually consisting of 10 to 20 layers of cells (Figures 19 and 20).

As already stated, in the young material there were no distinct visible histological modifications delimiting this zone. In somewhat mature material, however, the cells constituting this zone were found to be relatively smaller in size, isodiametric in shape, having denser protoplasmic contents and lacking intercellular spaces, as compared to the cells of the adjoining tissues (Figure 21).

Fig. 22. Longitudinal section of a pedicel, showing the actual separation along the distal face of the abscission zone.

Fig. 23. Longitudinal section of a pedicel showing the separation taking place in C (cortex) and P (pith), but not in VB (vascular bundle) elements.

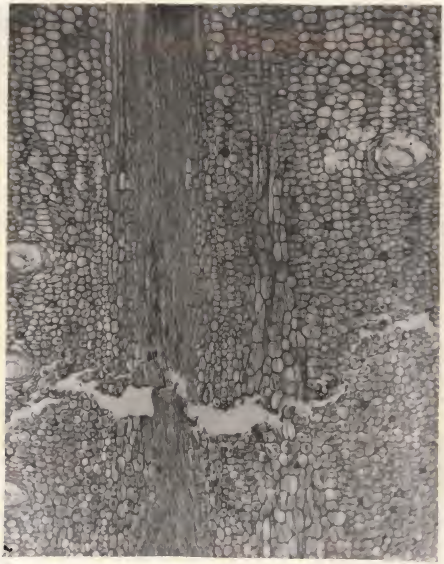

Fig. 24. Longitudinal section through the basal end of a pedicel, showing abscission taking place in an irregular line.

2. The Process of Separation

The actual separation occurred along the distal face of the abscission zone. Ordinarily one to five tiers of cells, two to three layers below the upper end of the abscission zone, were involved in the process of separation (Figure 22). The cells in the abscission zone began to separate in an irregular line. The path of the abscission is determined by the resistance offered by the different tissues to process of separation.

The process of separation most probably started in the cortex and progressed inwardly and outwardly, and involved all the tissues across the pedicel except vessels and fibers. They appear to be broken quite mechanically. This break is probably brought about by the weight of the developing ovary and by the environmental factors such as wind and rain (Figures 23 and 24).

The walls of all the cells of the abscission zone showed chemical alteration. The greatest alteration took place in the cells which were actually involved in separation. These changes could be detected by chemical means. The cells in the abscission zone of pedicels just prior to abscission showed marked inability to hold methylene blue stain, when the sections were stained over night. With the progress in separation process the cells in this zone were stained more deeply red with Ruthenium red. The vascular bundle elements in the abscission zone gave comparatively very light staining with safranin, indicating lesser lignification in this region. Tests for starch with I_2.KI showed that the tissues on

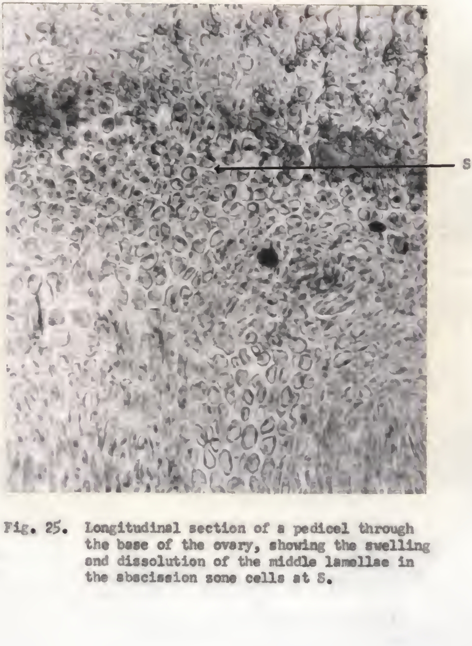

Fig. 25. Longitudinal section of a pedicel through the base of the ovary, showing the swelling and dissolution of the middle lamellae in the abscission zone cells at S.

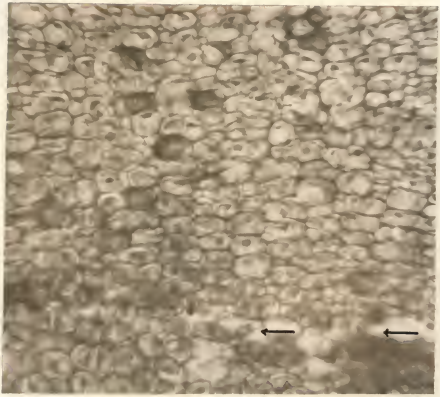

Fig. 26. Longitudinal section, showing part of the abscission zone, with dissolution of middle lamellae and partial digestion of cell walls indicated.

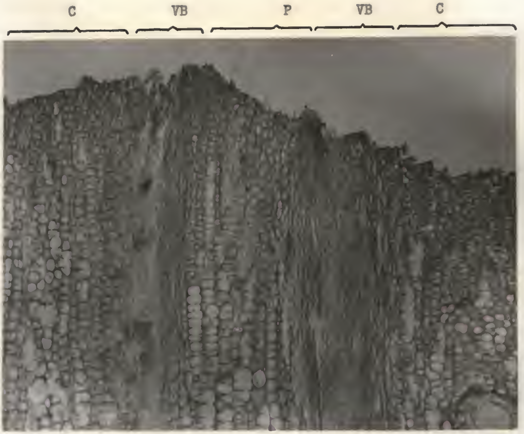

Fig. 27. Longitudinal section of a pedicel, soon after separation. C (cortex), VB (vascular bundle elements), P (pith).

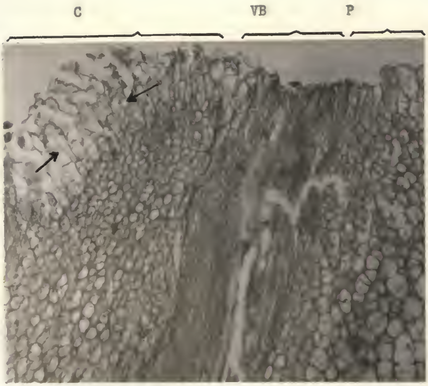

Fig. 28. Longitudinal section of a pedicel after fruit fall. Arrows show the area of secondary cell division which developed after fruit fall. This loose mass of cells and the suberization of distal tiers of cells in the cortex and pith tissues in the abscission zone, probably function as primary protective layer.

either side of the abscission zone were rich in starch prior to the abscission process. However, with the initiation of the abscission process, the cells in the abscission zone were found to be plentifully supplied with starch, while the adjoining tissues were found devoid of it.

The first step toward separation was swelling of the middle lamellae and of the outer portions of the cell walls themselves (Figure 25). The dissolution of the middle lamellae and of the gelantinized walls completed the separation process (Figure 26).

By this disorganization, the cells in the separation layer became isolated and free from one another, leaving them bounded by only delicate, tertiary membranes.

As already stated, in many cases pedicels remain attached to the main branches after the fruit fall. A mass of loose tissue was usually found on such areas indicating that the cells in the abscission zone remain active after fruit fall and resume growth and active division. The loose mass of cells thus formed probably serves to prevent the excessive loss of water and the entrance of pathogenic organisms. The tests for suberin with Sudan III showed that a few distal layers of cells in the abscission zone became suberized prior to fruit fall. This deposition of suberin and the formation of a loose mass of cells constitute a primary protective layer (Figures 27 and 28).

3. Periodicity of Fruit Abscission

Fall of fruit may be considered a continuous process. However, during the course of the development of fruit, several periods of

intense fruit abscission are quite noticeable. In Hamlin oranges, three such waves of dropping were quite pronounced. The first wave lasted for a month or so after full bloom. The second period of drop was from early May to the middle of June. And the third wave of drop was observed from late August onward.

The dates of collecting the samples, the total number of pedicels collected and examined, and the number of pedicels showing varying degrees of the separation process for the year 1955 are given in Table 1.

Similarly details concerning collections of samples and the results obtained through anatomical studies for the 1956 crop season are embodied in Tables 2 and 3. Figure 29 shows the periodicity of abscission graphically.

4. Abscission as Related to the Position of the Fruit in the Inflorescence

The data given in Tables 2 and 3 and the graphical presentation in Figure 29 suggest that the position of the fruit in the infloresence does not have any marked influence on the amount of abscission of fruit. Out of the 450 terminal and 450 lateral pedicels examined, 216 terminal and 237 lateral pedicels showed varying degree of separation taking place in the abscission zone.

While the terminal fruit showed slightly smaller tendency to abscission than the lateral fruit closest to it, the difference is probably too small to have significance.

Table 1.

ABSCISSION OF HAMLIN ORANGES DURING THE SEASON OF 1955, AS SHOWN BY THE NUMBER OF PEDICELS WITH SEPARATION OF VARYING DEGREE IN THE ABSCISSION ZONE.

Date of Sampling	April			May			June			July			August			September		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1st.	15	12	80	15	13	87	15	13	87	15	3	20	15	3	20	15	6	40
8th.	15	8	53	15	13	87	15	14	93	15	2	13	15	4	27	15	6	40
15th.	15	6	40	15	12	80	15	6	40	15	1	7	15	3	20	15	5	33
23rd.	15	8	53	15	12	80	15	3	20	15	3	20	15	5	33	15	7	46
Total	60	34		60	50		60	36		60	9		60	15		60	24	
Average			57			83			60			15			25			40

Grand Totals - A 360
B 168
C 47

Key

A. Total number of pedicels examined
B. Number of pedicels showing separation
C. B as percentage of A.

Table 2.

EFFECT OF INFLORESCENCE POSITION ON ABSCISSION OF HAMLIN ORANGES DURING THE SEASON OF 1956 AS SHOWN BY THE NUMBER OF TERMINAL PEDICELS WITH SEPARATION OF VARYING DEGREE IN THE ABSCISSION ZONE.

Date of Sampling	February			March			April			May			June			July			August			September		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1st.				15	12	80	15	14	93	15	6	40	15	10	67	15	1	7	15	2	13	15	7	47
8th.				15	12	80	15	13	87	15	6	40	15	12	80	15	0	0	15	3	13	15	6	40
15th.	15	8	53	15	13	87	15	5	33	15	7	47	15	4	27	15	3	20	15	6	40	15	7	47
23rd.	15	12	80	15	15	100	15	6	40	15	10	67	15	3	20	15	3	20	15	6	40	15	4	27
Total	30	20		60	52		60	38		60	29		60	29		60	7		60	17		60	24	
Average			67			87			63			48			48			12			28			40

Grand Totals - A 450
B 216
C 48.0

Key
A. Total number of pedicels examined
B. Number of pedicels showing separation
C. B as percentage of A

Table 3.

EFFECT OF INFLORESCENCE POSITION ON ABSCISSION OF HAMLIN ORANGES DURING THE 1956 SEASON AS SHOWN BY THE NUMBER OF LATERAL PEDICELS WITH SEPARATION OF VARYING DEGREE IN THE ABSCISSION ZONE.

Date of Sampling	February			March			April			May			June			July			August			September		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1st.				15	12	80	15	15	100	15	6	40	15	13	87	15	2	13	15	3	20	15	7	47
8th.				15	14	93	15	14	93	15	5	33	15	10	67	15	3	20	15	5	33	15	8	53
15th.	15	10	67	15	14	93	15	7	47	15	8	53	15	6	40	15	4	27	15	6	40	15	4	27
23rd.	15	11	73	15	15	100	15	7	47	15	10	67	15	4	27	15	2	13	15	7	47	15	5	33
Total	30	21		60	55		60	43		60	29		60	33		60	11		60	21		60	24	
Average			70			92			72			48			55			18			35			40

Grand Totals - A 450
B 237
C 52.6

Key
A. Total number of pedicels examined
B. Number of pedicels showing separation
C. B as percentage of A

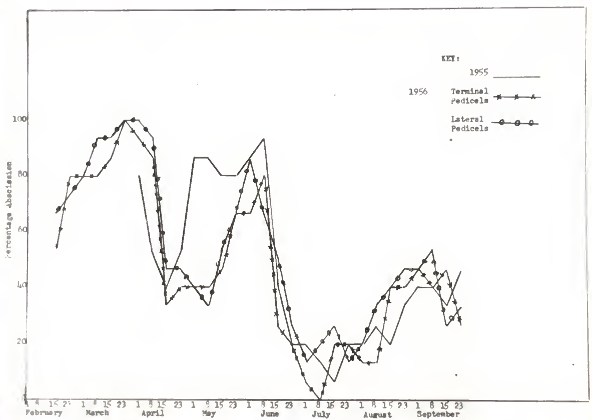

Fig. 29. Abscission of Hamlin oranges during the seasons of 1955 and 1956.

C. Results of Tests on Effect of Different Treatments of Explants on Abscission.

The details and the results of various treatments on the rate of abscission in Hamlin oranges are embodied in Tables 4 to 8. The data are presented graphically in Figures 30 to 34.

The results of these experiments clearly show that even the lowest concentrations of sucrose, 2,4-D, indoleacetic acid, and leaf and seed extracts have definite abscission-inhibiting effects.

Due to the tremendous difference between field conditions and the laboratory environments, different results might be expected under field conditions.

TABLE 4

THE EFFECT OF SUCROSE TREATMENT ON RATE OF ABSCISSION OF PEDICELS.

Time in Days	Number of Explants Showing Abscission with Various Concentrations of Sucrose				
	20%	10%	5%	1%	No Treatment
1					
2					
3					
4				1	2
5		1	1	2	3
6	1	.	3	2	3
7	2	3	.	1	2
8	2	2	3	1	
9	2	.	2	1	
10	.	2	1	2	
11	3	2			
12					
13					
14					

TABLE 5

THE EFFECT OF 2,4-D TREATMENT ON RATE OF ABSCISSION OF PEDICELS.

Time in Days	Number of Explants Showing Abscission with Various Concentration of 2,4-D			
	40 ppm.	20 ppm.	10 ppm.	No Treatment
1				
2				
3				
4				1
5				4
6			1	5
7			.	
8	1		2	
9		1	3	
10		2	1	
11	1	.	1	
12	3	3	1	
13	3	1	1	
14	1	2		

TABLE 6

THE EFFECT OF INDOLEACETIC ACID TREATMENT ON RATE OF ABSCISSION OF PEDICELS.

Time in Days	Number of Explants Showing Abscission with Various Concentrations of Indoleacetic Acid				
	10,000 ppm.	1,000 ppm.	100 ppm.	50 ppm.	No Treatment
1					
2					
3					1
4			2	1	3
5			1	2	3
6		2	.	2	3
7		2	4	5	
8		.	2		
9		1	1		
10	1	3			
11	2	1			
12	.	1			
13	1				
14	1				

TABLE 7

THE EFFECT OF CELL SAP (EXTRACTED FROM HAMLIN LEAVES) TREATMENT ON RATE OF ABSCISSION OF PEDICELS.

Time in Days	Number of Explants Showing Abscission at Different Concentrations			
	Full Strength (or Undiluted)	Diluted One-Half	Diluted One-Fourth	No Treatment
1				
2				
3				
4				4
5			1	2
6			2	2
7		2	3	2
8		1	.	
9	1	.	2	
10	.	1	1	
11	2	.	1	
12	.	3		
13	2	3		
14	2			

TABLE 8

THE EFFECT OF TREATMENT WITH SEED EXTRACT (OF HAMLIN ORANGES) ON THE RATE OF ABSCISSION OF PEDICELS.

Time in Days	Number of Explants Showing Abscission at Different Concentrations		
	Full Strength (or Undiluted)	Diluted One-Half	No Treatment
1			
2			
3			1
4			3
5			3
6			.
7			3
8			.
9	2	3	
10	2	2	
11	.	.	
12	3	.	
13	1	3	
14		1	

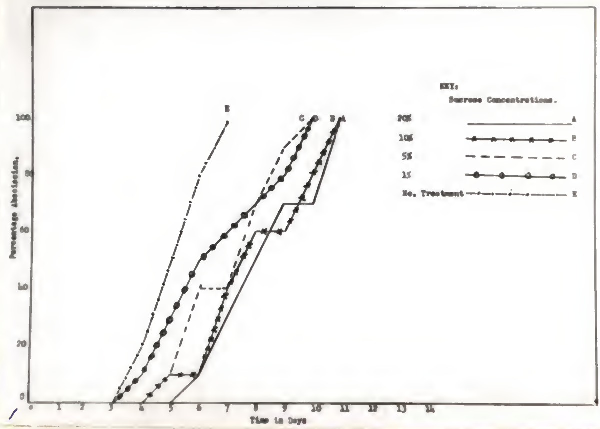

Fig. 30. Effect of sucrose treatments on rate of abscisson of pedicel explants.

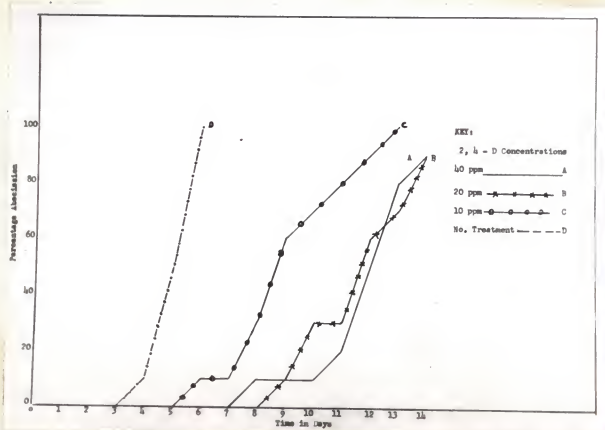

Fig. 31. Effect of 2,4-D treatments on rate of abscission of explant pedicels.

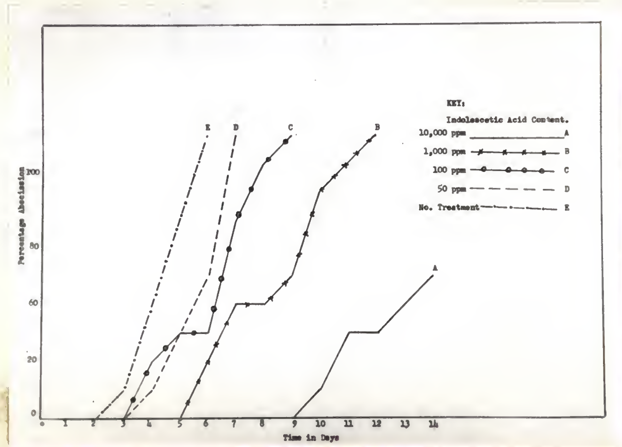

Fig. 32. Effect of indoleacetic acid treatments on rate of abscission of explant pedicels.

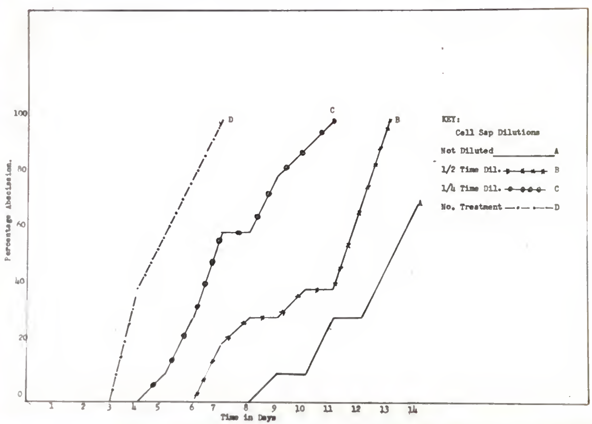

Fig. 33. Effect of expressed sap of Hamlin orange leaves on rate of abscission of explant pedicels.

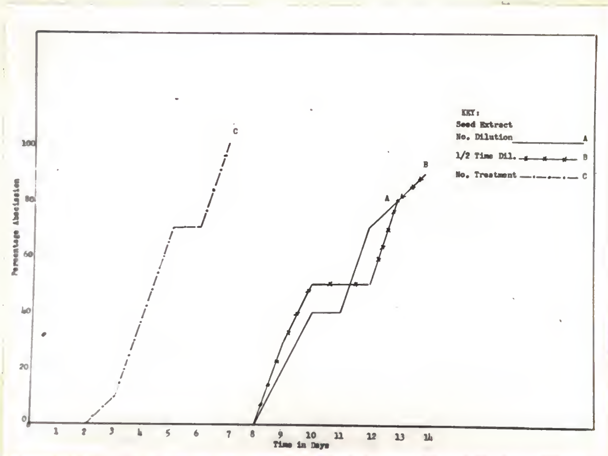

Fig. 34. Effect of extracted sap from Hamlin orange seeds on rate of abscission of explant pedicels.

V. DISCUSSION

A. Anatomy of Abscission Zone

The present anatomical studies suggest that fruit drop in Hamlin oranges is associated with the formation of a specialized zone, the abscission zone. This zone is characterized by a reduction in the diameter of the pedicel, and the reduction in amount of tissues. The cells in this zone were found invariably to be smaller in size as compared to the cells constituting tissues on both sides of the zone. No cell division was noticed in this zone. It is presumed that the cells constituting the tissue are those which did not increase in size while the cells in the adjoining tissues did during the course of the development. Hodgson (90) similarly did not find any cell division in the abscission zone while working on foliar abscission in Valencia orange. However, work of MacDaniels (60) and McCown (63, 64) with apples, and of Lloyd (57) with Mirabilis jalapa, gave evidence of such cell division associated with abscission.

In mature pedicels the abscission zone was invariably noticed without difficulty in all the specimens examined due to histological differentiation of the cells making up this zone. In young material, where such histological differentiation was not so distinct, this zone could still very easily be located through chemical tests. The presence of this zone in almost all the pedicels examined suggests that the abscission zone itself is preformed and only the initiation

of the separation process is influenced by various climatic, nutritional and environmental factors.

Early in the season the abscission of young fruits was invariably near the base of the pedicel. From early May onward most of the fruits were seen to abscise at the base of the ovary. Most of the mature specimens studied showed abscission taking place in both regions, with partial and incomplete separation near the base of the pedicel, and a very advanced stage of separation at the base of the ovary. This suggests that most probably separation is initiated in two zones, but the rate of separation near the base of pedicels is slower, probably due to the presence of more strengthening tissues at this place. These findings are in agreement with the results reported by Baird and Laurie (5), Hodgson (40), MacDaniels (60), and McCown (63, 64).

B. The Separation Process.

The process of actual separation occurs in two steps. The first was the marked swelling and gelatinization of middle lamellae and cell walls, while the second step was the dissolution of middle lamellae and digestion of the outer cell walls.

According to Allen (4), Carre (13), Jones (43), McCown (64), and many other workers, the middle lamella consists largely of pectic compounds which appear to be a mixture of calcium and magnesium pectates and pectose, whereas the adjoining layers of the primary wall as well as the layers of the secondary wall contain cellulose and are

largely composed of this substance. Besides cellulose they may contain varying amounts of pectic compounds, hemicellulose, lignin, silica, etc.

Gaddum (24) reported three principal pectic compounds in citrus fruits: pectic acid, pectin, and protopectin. All of these compounds are believed to be important constituents of cell walls and middle lamellae and have been described as gelatinizable substances.

Sampson (77) theorized that the abscission of leaves in Coleus blumei is a result of the conversion of cellulose into pectose which is further transformed to pectin and pectic acid, leading to the formation of an excess amount of pectic acid over that of the available calcium sufficient to maintain the solidity of the middle lamella of the cell walls of the abscission layer. McCown (65) similarly reported that the abscission of apple fruits is the result of the gradual disintegration of the cellulose cell walls. At the same time the character of the middle lamella changes. This he concluded from the swelling of the middle lamella and from greater intensity of staining with Ruthenium Red. He asserted that the separation of the cells is brought about by the dissolution of the pectic compounds which is the result of the conversion of insoluble forms to soluble forms of pectic materials. He also reported that the fibers and xylem vessels in the path of abscission are ruptured by mechanical stress and are not involved in changes accompanying abscission.

In the light of these findings it is presumed that the separation in the abscission zone in Hamlin orange is brought about by the conversion of the insoluble pectic compounds into soluble pectic

compounds. These changes are probably brought about through enzymatic action. Resistance of tracheids and fibers to abscission is probably due to some lignification of these tissues.

Davidson and Willaman (18) and various other workers recognized protopectinase, pectinase, and pectase associated with the conversion of protopectin into pectin, pectic acid into galacturonic acid, and pectin into pectic acid, respectively. Jones (43) isolated a cytolytic enzyme from Bacillus carotovorus which he recognized as pectinase, and concluded that this enzyme attacks the middle lamellae, causing first a gelatinization followed by dissolution, resulting in the separation of the cells in the tissue attacked.

The reason for the presence of starch in the abscission zone after it disappeared in the adjoining tissues, may be attributed to the changes in the cell wall incident to the abscission phenomenon. Probably these changes hinder the translocation of the products of starch hydrolysis to adjacent tissues.

A mass of loose cells which made its appearance on the scar after fruit fall and the suberization of the distal tiers of cells in the abscission zone, probably serve as a primary protective layer prior to periderm formation. A similar loose mass of cells was noticed by Hodgson (40), McCown (64), and Namikawa (71). They proposed that the function of such a mass of loose cells is to provide protection against excessive transpiration and entrance of pathogenic organisms.

C. Periodicity of Abscission

Three pronounced waves of fruit drop were observed in Hamlin variety of oranges grown under Florida conditions. The anatomical studies also showed that pedicels examined during certain parts of the year showed more evidence of separation in the abscission zone than in other parts of the year. The first wave of fruit fall, which coincided with petal fall, consisted of pistils, other flower parts, and young developing fruits. Most of the fruits lost in this drop showed distorted styles, and one of the several causes of this drop apparently is the resulting defective pollination and failure of fertilization. The other cause of this drop is undoubtedly competition for food, because it is impossible for a tree to bring to maturity all the bloom it puts forth. The climatic conditions prevailing at the time of fruit set also determine the amount of the drop. Heinicke (37) and Murneek (67), working with apples attributed the major part of this drop to poor pollination and failure of fertilization. They arrived at this conclusion due to the fact that fruit lost in this drop showed comparatively fewer seeds. However, they also noticed fruits lost in this drop which contained a relatively large number of seeds, so they added that such a loss might have been caused by other climatic, nutritional, and pathological agencies, such as winds, frost, hail, diseases and insects, etc. But there was evidence of the drop even in the absence of such destructive agents. Their further studies suggested that the amount of food and water which reaches the

individual flowers and fruits determines whether or not they will remain on the tree. If the sap supply is adequate, the flowers set fruit and the fruits mature, notwithstanding the fact that they may have fewer seeds.

Similarly the work of Haas (34, 35) with citrus supports the idea that production of flowers results into a severe tax on the food reserves of the tree and that after excessive production of flowers the vitality of the tree is considerably reduced. Thus competition for food makes the excessive fruit to fall.

The second drop or the June drop was experienced from early May to the middle of June in 1955 and from late May to the middle of June in 1956.

Field observation showed that this drop was more intense during the hot dry spells experienced in these summer months. Studies of the meteorological data condensed and embodied in Table 9 revealed that the first half of May, 1955, was extremely hot and dry. A monthly total of 2.50 inches of rainfall was almost all received in the second half of the month, whereas, on the other hand the first half of May, 1956, was comparatively cool and wet as compared to the latter half. The evidence of the intensity of abscission in early May in 1955 and in the latter half of May in 1956 suggests that abscission of fruit during summer months is greatly influenced by temperature and moisture. Further perusal of the meteorological records shows that comparatively higher rainfall was received in months of July in both years. The abscission of fruit was at its lowest ebb during this month. It appears, therefore, that high temperatures accompanied

TABLE 9

MONTHLY MEAN MAXIMUM TEMPERATURE AND RAINFALL, AND THEIR RELATION TO ABSCISSION PHENOMENON OF HAMLIN ORANGES.

	1955			1956				
						Percentage of Pedicels with Abscission		
Month	Mean Maximum Temperature	Rainfall in Inches	Percentage of Pedicels with Abscission	Mean Maximum Temperature	Rainfall in Inches	Terminal	Lateral	Average
February				74.6	4.10	67	70	68
March				76.3	.88	87	92	89
April	83.3	1.25	57	80.0	3.51	63	72	68
May	91.2	2.50	83	89.9	4.12	48	48	48
June	89.9	7.04	60	84.0	11.44	48	55	52
July	92	11.92	15	91.9	5.55	12	18	15
August	93	5.19	25	92.4	7.88	28	35	32
September	91.3	4.04	40	87.96	3.12	40	40	90

by drought are detrimental, but are not so when there is much rainfall. Apparently high temperatures augment the rate of transpiration and when this occurs under drought conditions soil moisture becomes depleted. Uptake of water cannot keep pace with the rate of transpiration, and thus a water deficit develops in the leaves which is transmitted to other parts of the plant and serves as a stimulus for fruit abscission.

Coit and Hodgson (16) and Hodgson (41), working with navel oranges, reported withdrawl of water from fruit by the transpiring leaves under drought conditions. According to their hypothesis a diurnal decrease in water content of the fruits occurs during the afternoon and is accompanied by a considerable increase in the water deficit of the leaves. Negative pressures of considerable magnitude are found in the water columns of citrus trees, under conditions of high temperature and low soil moisture. They recorded the maximum dropping of the fruits to be most severe where the above-mentioned relations were most abnormal. Ziegler (97) in his irrigation studies with Marsh grapefruit, observed that in dry years when less than 20 inches of rainfall was received from January to June, the crop was decreased by some 20%. He emphasized the need of irrigation during these hot dry months under low rainfall conditions.

The fruits lost in preharvest drop were invariably found infected with <u>Alternaria citri</u> and there seems every likelihood that a varying percentage of fruit drop late in the maturing season is brought about by this fungus. The work of Coit and Hodgson (15, 16)

and Wager (90) showed the influence of this fungal attack on fruit loss late in the season. They described Alternaria citri as a weak parasite which infects the fruits early in the season, after the style has been shed, and remains quiescent in the young developing fruits, at which time the fruit is more or less resistant to the detrimental effects of this parasite. They asserted that with the decline in vigor, which occurs as the fruit approaches maturity, the fungus becomes active and exerts a deteriorating effect on the fruit. They theorized that excessive transpiration from the leaves causes water, together with enzymatic solution secretions by the fungus in the navel end of the fruit, to be drawn back through the vascular system of the young fruits to the pedicel thus providing the stimulus to abscission.

Hamlin oranges due to their different morphological characters should be less susceptable to the attack of Alternaria than Washington navel oranges. Nevertheless, most of the fruits lost late in the maturing season showed the incidence of black rot. It therefore seems very likely that part of the preharvest drop is brought about by Alternaria citri. Unfavorable climatic conditions responsible for the June drop are probably responsible for the other part of the preharvest drop. Decline of auxin in maturing fruits as postulated by Luckwell (59) may also be one of the causes for this late season drop.

D. Abscission as Related to the Position of the Fruit on the Tree.

The work of Skoog and Thimann (83) and Snow (85) suggests the apical dominance of the terminal bud over the lateral buds. In the present studies an attempt was made to find if some similar correlation exists between the position of the fruit on the floral axis and the incidence of abscission. Of 450 terminal and 450 lateral pedicels, 216 terminal and 237 lateral pedicels showed varying degrees of separation. The difference is so small that there does not seem to be any relation between the relative position of the fruit and the abscission phenomenon in Hamlin orange.

Coit and Hodgson (16) in their morphological studies also did not report any striking difference in the rate of fall of fruit as related to its position. They held the idea that terminal fruits are probably in a slightly better position to benefit from water supply, being located on the main axis which has a greater development of secondary xylem than the lateral pedicels.

E. Tests on Effect of Treatments of Explants on Abscission.

The results of the experiments designed to note the effect of the treatment of explants with different materials showed the definite abscission-inhibiting influence of the materials used. Untreated explants showed 100% abscission within a week, whereas a good percentage of the explants variously treated remained intact over a two-week's period. In case of the sucrose and 2,4-D experiments, the curves

representing the effect of different concentrations on the rate of abscission stood quite close and did not show much difference in the effectiveness of different concentrations, indicating that only minute quantities of such materials are needed to stimulate the reaction which results in the inhibition of abscission. Indoleacetic acid, which seemed most potent in decreasing the progress of separation, showed an apparent correlation between the concentration employed and the amount of inhibition of abscission.

The effects of the treatments with leaf and seed extracts were found to be rather similar to the results obtained through the use of indoleacetic acid and 2,4-D.

Gardner (26), Reece and Horanic (75), Sites (82), and Stewart and Klotz (87) tried various growth promoting substances for the control of preharvest drop in citrus on a field scale, and reported substantial success in control of this drop in Valencia and Pineapple oranges with 2,4-D, 2,4-5Tp and other related substances.

The results obtained in the laboratory cannot be expected to be identical with those observed in the field. However, the present findings clearly show the abscission-inhibiting effect of surose 2,4-D, indoleacetic acid, and leaf and seed extracts.

Carns et al. (12) in their study included the treatment with water immersion of the explants and reported an appreciable decrease of abscission by this treatment alone. In the present studies the various concentrations and the dilutions of the materials used were made in distilled water. Consequently a part of the abscission

control by these treatments should be attributed to the presence of water. The above authors concluded that the effect of water immersion is due to the fact that immersion checks the uptake of oxygen which results in the slowing down of respiration and thus inhibits abscission. Wildman and Bonner (95), working with spinach, reported that water immersion helps in delay of abscission, possibly by leaching some substance necessary for abscission or by releasing some substance which inhibits abscission from the bound state to the active state.

The results obtained by the use of sucrose are in confirmation of the results reported by Went and Carter (93). They showed definite decrease in the abscission of tomato flowers when plants grown under unfavorable conditions were sprayed with sugar.

It is common knowledge that girdling a stem leads to the accumulation of carbohydrates above the girdle and often retards the abscission of plant organs. This, too, suggests the pronounced effect of carbohydrates on abscission.

Kraus and Kraybill (48) also suggested that high carbohydrate content makes for continued development of the vascular strands of the pedicels and the strengthening of their connection with the spurs. However, a proper balance is needed between carbohydrates and nitrogen for such physiological action.

Many workers have reported that growth-promoting substances act primarily in a regulatory capacity in some phase of the carbohydrate metabolism in the plant. Mitchell and Whitehead (66), working with bean plants, suggested that introduction of growth-regulating

substances into leaves or cuttings induces a marked hydrolysis of starch. Sell et al. (80), while studying the changes in chemical composition of the stems of red kidney bean plants, found that treatment with 2,4-D accelerates the hydrolysis of starch to sugars and thus promotes mobilization of carbohydrates in tissues, and probably results in the decrease of abscission.

The results of the present studies do indicate a strong abscission-inhibiting effect of 2,4-D treatment, which may be due to its direct effect on cells or due to its activation of other growth-regulating compounds present in the tissues.

The results obtained by the use of indoleacetic acid are in line with the results reported by Gardner and Cooper (27), LaRue (49) McCown (65), and Myers (70), who used indoleacetic acid effectively to inhibit foliar abscission in coleus and fruit abscission in apples. Indoleacetic acid is believed to occur naturally as an auxin in plant tissues. Haagen-Smit et al. (32) were first successful in isolating it from developing corn seed. It is now known that indoleacetic acid is widespread in plants and is found in growing regions such as shoot tips and leaves. Young fruits and ovules are high in indoleacetic acid.

The treatment of explants with extracts obtained from leaves and seeds of Hamlin oranges demonstrated their abscission-inhibiting effect. LaRue (50) and many other workers have already shown that a bladeless petiole soon falls on account of the fact that they are deprived of the auxin produced in the leaf-blade. Similarly Luckwell (59) reported that the successive waves of fruit-drop in apple are correlated with temporary deficiencies in hormone production resulting

from developmental changes in the endosperm. In the light of these findings it seems most probable that the abscission-inhibiting effect of treatments with extracts of orange leaves and seeds is due to the presence of some naturally occurring hormone.

In the light of the present studies and of the findings reported by other workers on related problems, it can be concluded that fruit fall in the Hamlin oranges constitute true abscission. Fruit fall is brought about by the separation of cells in the preformed abscission zone under the influence of several climatic, nutritional, and environmental factors. The process of separation is probably effected by certain enzymes which promote the conversion of insoluble pectic compounds into soluble pectic compounds. This results in the swelling and then dissolution of the middle lamella and outer cell walls, thus freeing the cells from one another. Unfavorable climatic conditions, competition for food and water, and incidence of pests and diseases result in waves of intense fruit abscission, coinciding with such unfavorable conditions. The abscission phenomenon is most probably closely related with some naturally-occurring growth hormones in the plant tissue, depletion of which results in an increased rate of abscission.

Ordinarily a satisfactory harvest should be expected after a normal first drop. From the grower's point of view, prevention of abscission of fruit is not important at this stage of the fruit development. However, in certain years of hot dry summers, a considerable reduction in the amount of harvest will result through

excessive shedding of fruit unless sparse natural precipitation is supplemented with judicious irrigation.

Preharvest drop is of great concern to growers, since an almost mature crop is lost through this drop shortly before harvest. Alternaria citri seems associated with a varying percentage of this drop. In view of the fact that only a part of this drop is due to fungal attack, some sort of spraying program to reduce that aspect of fruit fall may not pay its way. Drought conditions in some years may also be one of the causes, but irrigation at this time of the year for Hamlin oranges will adversely affect the percentage of total soluble solids and therefore cannot be recommended. Spraying with some potent growth-regulating substance or hormone may be the answer to the problem to some extent, and this is already being practiced in various ways with many horticultural crops.

VI. SUMMARY

1. Investigations into various aspects of fruit drop in citrus were carried out in the young grove at the University of Florida.
2. Three apparently uniform Hamlin orange trees of the same age and under similar cultural practices furnished the experimental material.
3. The anatomical studies revealed that the abscission zone in the pedicels of Hamlin oranges is preformed.
4. The cells in the abscission zone were found to be relatively small in size as compared to cells in adjacent tissue. They were isodiametric in shape, having dense protoplasmic contents. Intercellular spaces were lacking.
5. The abscission of fruit early in the season was invariably near the base of the pedicel, while after early May, most of the fruits abscissed at the base of the ovary.
6. The separation of cells in a few tiers of cells close to the distal end of the abscission zone, which itself consisted of 10 - 20 tiers of cells, is influenced by climatic, nutritional, and environmental conditions, such as pollination, fertilization, temperature, moisture, incidence of pests and diseases, etc.
7. The first indication of separation in the abscission zone was the swelling of the middle lamellae, followed by their dissolution and that of the outer cell walls. The process involved in this separation is the conversion of insoluble pectic compounds into soluble ones.

8. After fruit fall a loose mass of cells which forms on the scar, and the suberization of distal tiers of cells in the abscission zone, probably function as protective layers.
9. Three pronounced waves of fruit drop occurred. The first, coinciding with petal fall, was evidenced by the drop of the ovary with other floral parts. There is strong indication that the second, or June drop is largely the result of high temperatures accompanied by severe drought conditions. Preharvest drop is probably due to the decline in vigor of fruit as maturity approaches, and to the activity of *Alternaria citri*.
10. The relative position of the fruit on the axis did not show any marked influence on the rate of abscission of fruits.
11. The abscission-inhibiting effect of sucrose, 2,4-D, indoleacetic acid, and leaf and seed extracts in the laboratory tests suggests that the abscission phenomenon is probably closely related to some naturally occurring growth-promoting hormone in the plant tissues.

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BIOGRAPHICAL SKETCH

Saeed Ahmed Chaudhri was born on June 18, 1923 at Gharyala, Punjab, India. He received his secondary school education at D. B. High School, Patti, Punjab, and passed the Matriculation Examination of the Punjab University, Lahore, in 1942. He continued his education at the Punjab Agricultural College, Lyallpur and was awarded the degree of Bachelor of Science in Agriculture in 1946, and of Master of Science in Agriculture in 1948, from the Punjab University. Immediately after completion of his studies he was employed as Research Assistant with the Fruit Section of the Agriculture Department of Punjab, Pakistan. During the tenure of his service in the Agriculture Department, he worked on various research projects and also helped in the teaching and extension activities of the section. In 1954 he was selected by the United States Education Foundation in Pakistan as Fulbright Travel Grantee to enable him to obtain advanced training in Horticulture at the University of Florida, Gainesville, Florida, U. S. A.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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